Comparative Genomic Hybridization and Loss of Heterozygosity Analyses Identify a Common Region of Deletion at 15q11.1–15 in Human Malignant Mesothelioma

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ABSTRACT

Comparative genomic hybridization analysis was performed to identify chromosomal imbalances in 24 human malignant mesothelioma (MM) cell lines derived from untreated primary tumors. Chromosomal losses accounted for the majority of genomic imbalances. The most frequent underrepresented segments were 22q (58%) and 15q11.1–21 (54%); other recurrent sites of chromosomal loss included 1p12–22 (42%), 13q12–14 (42%), 14q24–qter (42%), 6q25–qter (38%), and 9p21 (38%). The most commonly overrepresented segment was 5p (54%). DNA sequence amplification at 3p12–13 was observed in two cases. Whereas some of the regions of copy number decreases (i.e., segments in 1p, 6q, 9p, and 22q) have previously been shown to be common sites of karyotypic and allelic loss in MM, our comparative genomic hybridization analyses identified a new recurrent site of chromosomal loss within 15q in this malignancy. To more precisely map the region of 15q deletion, loss of heterozygosity analyses were performed with a panel of polymorphic microsatellite markers distributed along 15q, which defined a minimal region of chromosomal loss at 15q11.1–15. The identification of frequent losses of a discrete segment in 15q suggests that this region harbors a putative tumor suppressor gene whose loss/inactivation may contribute to the pathogenesis of many MMs.

INTRODUCTION

MM is an asbestos-related malignancy that arises primarily from the surface serosal cells of the pleural, peritoneal, and pericardial cavities. An increased incidence of MM has been recognized worldwide, and this increase is predicted to continue until the year 2020 (1). MM is characterized by a long latency, typically 20–40 years, from the time of exposure to asbestos to clinical diagnosis, suggesting that multiple, cumulative somatic genetic events are required for tumorigenic conversion of a mesothelial cell.

Early studies performed with conventional chromosomal banding techniques revealed numerous karyotypic alterations in most MMs (2–5). Among the changes are recurrent deletions of discrete segments within chromosome arms 1p, 3p, 6q, 9p, and 22q (5). Subsequent studies of allelic loss in MMs have documented high frequencies of LOH at 1p22, 3p21, and 6q and homozgyeous deletions involving 9p21–22 in MM cell lines (reviewed in Ref. 6). Furthermore, the TSGs CDKN2A/p16 (located at 9p21) and NF2 (located at 22q12) have been shown to be frequently altered in MM (7–9).

CGH analysis is a valuable technique for whole genome scanning. This procedure permits the identification of chromosomal imbalances (gains, losses, or amplification of DNA sequences) in entire tumor genomes (10). Importantly, CGH has facilitated the identification of consistent sites of chromosomal imbalance in a wide variety of solid tumors. Thus far, CGH analysis of MM has been limited to a series of tumors from Finland in which recurrent losses from 4q, 6q, 9p, 13q, 14q, and 22q were documented (11, 12). However, more than 30% of these cases showed a normal CGH pattern, presumably due to significant contamination of tumor specimens by normal stromal cells.

In this investigation, CGH studies were carried out on tumor cell lines derived from 24 primary MMs. Some of the regions of copy number decreases identified in this investigation are consistent with previous karyotypic and LOH studies of MM. However, we also report several additional recurrent imbalances, prominent among these being the frequent underrepresentation of 15q, which was confirmed by LOH analysis as a discrete site of interstitial deletion from 15q11.1–15, suggesting that the loss and/or inactivation of a putative TSG in this region may contribute to the pathogenesis of many MMs.

MATERIALS AND METHODS

Cell Lines and Tumor Specimens. Tumor specimens were surgically resected primary MMs. Tumor cell lines were established from primary MM cultures as described elsewhere (5). All MM cases were newly diagnosed, untreated patients.

CGH. Genomic DNAs were isolated from MM cell lines, tumor specimens, and normal blood by standard methods. Protocols for labeling tumor and normal reference DNAs, capture of gray-level fluorescence images with a cooled charge-coupled device camera (Photometrics, Tucson, AZ), and digital image analysis were as described previously (13, 14). For each tumor specimen, the mean values of individual ratio profiles were calculated from at least seven metaphase spreads. Averaged values were plotted as profiles alongside individual chromosome ideograms indicate different threshold values between tumor DNA and normal reference DNA. The line on the left corresponds to a threshold value of 0.75, which would exist if 50% of the cells from a near-diploid tumor had monosomy of a given chromosome. The center line indicates a balanced state. The line on the right represents a threshold value of 1.25, which would occur if 50% of the cell population exhibited trisomy for that chromosome. Overrepresentation defined by a sharp peak was considered indicative of DNA sequence amplification.

PCR and LOH Analysis. Altogether, 16 microsatellite markers distributed along chromosome arm 15q were examined. Primer pairs used for PCR amplification of dinucleotide repeat markers were obtained from Research Genetics (Huntsville, AL). The relative order of these markers and the chromosomal localization of the microsatellites were obtained from the Genome Database (Johns Hopkins University). The PCR protocol was as described previously (15). In each case, 20 ng of genomic DNA were amplified in a 10-μl reaction volume using a MJ Research PTC-100 programmable thermal controller. Amplification conditions consisted of an initial denaturation for 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 2 min at either 55°C or 57°C, and 2.5 min at 72°C and by a 5-min extension at 72°C. PCR products were diluted 1:1 with a 95% formamide gel-loading buffer; 7 μl of each PCR product were separated on a 6%
polyacrylamide sequencing gel. Gels were dried at 80°C under a vacuum and subjected to autoradiography at 270°C for 16–72 h. LOH was scored when there was constitutional heterozygosity in the control DNA and complete loss of an allelic band in the tumor cell line DNA.

RESULTS

CGH analysis was performed on 24 MM cell lines. In three cases, CGH analysis was also carried out on the tumor specimens from which the cell lines were derived. Among the latter three cases, one tumor sample showed a CGH profile very similar to that observed in the corresponding tumor cell line, except that the CNAs were accentuated more in the cell line than in the tumor specimen (Fig. 1). The other two tumor specimens showed normal CGH profiles, presumably due to significant contamination by stromal cells.

All 24 MM cell lines exhibited multiple genomic imbalances (range, 6–25/case; mean number, 13.1/case), with every chromosome involved at least once in a CNA. A schematic summary of all CNAs is shown in Fig. 2. Chromosomal losses were more common than gains. The chromosome arms most often underrepresented were 22q (58%) and 15q (54%). Underrepresentation of 22q involved the entire long arm in each case affected, whereas CNAs of chromosome arm 15q were often interstitial losses with the SRO at 15q11.1–21 (Fig. 2). Representative profiles showing partial losses of 15q are depicted in Fig. 3. Other commonly underrepresented segments were 1p12–22 (42%), 14q24–qter (42%), 13q12–q14 (42%), 9p21 (38%), and 6q25–qter (38%). In addition, losses involving 8p22–pter, 10q23–25, 16p11.1–13.1, and 18q22–qter were each observed in 33% of the cases.

The most frequently overrepresented chromosome arm was 5p (54%). Other commonly overrepresented chromosomal segments included 5q14–23 (42%), 7p21–pter (33%), 7q31–35 (33%), and 8q24 (33%). Amplification of 3p12–13 was observed in two MMs; a gain of this same chromosomal region was seen in another five cases (Fig. 4). Amplification of 6q12–14, 6q25, and 20q13.3 was each detected in
a single case. Overrepresentation of 7p (SRO at 7p21–pter) was more common in cell lines from sarcomatous and biphasic tumors [6 of 12 cell lines (50%)] than in those from epithelial tumors [2 of 12 cell lines (17%)].

Overall, the CGH analyses revealed underrepresentation of 15q in 13 cases. CGH has limited resolution (5–15 megabases; Ref. 14), and the distal boundaries of a chromosomal loss are based on the inflection of the ratio profile and therefore may not be precise. To map the region of 15q loss in MM with higher resolution, we performed LOH analyses with a panel of polymorphic DNA markers. Matched normal DNA was available for 8 of the 13 cases with an underrepresentation of 15q, and each of these 8 cases exhibited LOH at multiple loci. Among these eight cases, two showed LOH at all informative loci, suggesting a whole chromosome loss in each case; interstitial deletions were observed in the remaining six MMs (Fig. 5A). All of the interstitial deletions extend from and include D15S986, D15S122, and/or D15S128 (located at 15q11.1–11.2), the most proximal markers used. The SRO of deletions defines a distal boundary of the minimally deleted region at D15S1012 (15q15) based on the deletion in case 7 (Fig. 5A). Examples of allelic loss from the 15q11.1–15q15 region are shown in Fig. 5B. In five of the eight cell lines with LOH from 15q, allelic losses from the same region were confirmed in DNA from tumor tissue, i.e., DNA from the tumor specimen exhibited a >50% reduction in the intensity of allelic bands, corresponding to those unequivocally lost in the matched cell line. In the remaining three cases, LOH from 15q was not obvious in the tumor specimen, possibly due to significant contamination by normal stromal cells, which is a common feature in MM (16).

Among the 11 cases that did not show underrepresentation of 15q by CGH analysis, matched normal DNA was available in 4 cases, 2 of which displayed allelic losses at all informative loci. Such losses may have escaped detection by CGH if the remaining homologue was duplicated via a mitotic nondisjunction event.

DISCUSSION

Some of the regions of copy number decreases (i.e., 1p, 6q, 9p, and 22q) reported here have previously been shown to be common sites of karyotypic and allelic loss in MM (2–6). Underrepresentation of 22q was the most prominent feature in our series. Consistent with this finding, karyotypic studies have shown that loss of chromosome 22 is the most frequent numerical abnormality in MM (2–5). Frequent mutations of a target locus at 22q12, i.e., the TSG NF2, have been documented in primary tumors and cell lines from patients with MM (8, 9). We previously reported karyotypic and allelic losses from 1p13–22 in many MMs (4, 5, 17), suggesting that alterations of a
putative TSG located in this region frequently contribute to the pathogenesis of this malignancy. Cytogenetic deletions of 6q are also common in MM (2, 3, 5, 18), and LOH analysis has revealed several discrete regions of deletion of 6q in MM, suggesting the involvement of multiple TSGs within this chromosome arm (15). Karyotypic analysis has documented frequent losses of 9p21–22 in MM (5), and gene dosage studies demonstrated a high incidence of homozygous deletions within this region (6). Subsequent work revealed frequent homozygous deletions of the TSG CDKN2A/p16 in MM tumor specimens and cell lines (7).

In addition to the losses mentioned above, our CGH analyses have identified specific sites within 8p, 13q, 14q, and 15q that are commonly underrepresented in MM. Previous karyotypic studies have documented whole or occasionally partial losses of chromosomes 13 in 14 in a minority of MMs (2–5), and loss of 8p has also been noted in a few cases (2, 3, 5). Although loss of the entire chromosome 15 or variable segments of 15q has been observed in some cases of MM (2, 3, 5), a consistent site of deletion from 15q has not been described to date. Thus, the studies presented here further demonstrate the value of CGH analysis in facilitating the identification of common sites of chromosomal imbalance that may have been overlooked in earlier cytogenetic studies.

Our CGH studies detected underrepresentation of 15q in a significant proportion (54%) of cases, and LOH analysis demonstrated allelic losses from 15q, with the SRO of deletions residing at 15q11.1–15. Losses overlapping this same region have also been observed in several other tumor types, including primary prostate cancers (19), ovarian cancers (20), parathyroid adenomas (21), and metastatic tumors of the breast, lung, and colon (22). Collectively, these data suggest that loss and/or inactivation of a putative TSG in 15q11.1–15 contributes to the development or progression of MM as well as other types of tumors. RAD51, which is located at 15q15.1, is a potentially relevant gene in this region. RAD51 is the human homologue of the bacterial RecA gene whose product participates in the repair of double-strand breaks and in chromosomal disjunction. Mutations in the mouse Rad51 gene have been associated with severe chromosomal loss in actively dividing cells (23), and deletions and numerical losses appear to be the predominant form of genomic imbalance in MM. Interestingly, our CGH analyses also reveal recurrent losses from 13q12–14 (Fig. 2), the location of the BRCA2 gene, whose product is an essential co-factor in RAD51-dependent DNA repair of double-strand breaks (24).

The short arm of chromosome 5 was the most frequently overrepresented site observed in this series of MMs. CGH analyses have also documented frequent overrepresentation of 5p in lung cancer (25) and in head and neck tumors (26). Candidate gene SKP2 (5p13) encodes a protein associated with the cyclin-dependent kinase 2/cyclin A complex. This protein has been found to be essential for S-phase entry (27).

Many of the genomic imbalances identified in this investigation are in agreement with CGH findings in Finnish MM (11, 12). Underrepresentation of portions of 6q, 9p, 13q, and 14q and overrepresentations of 7p and 7q were frequently observed in our cases and in those from Finland. In addition, losses from 1p and 22q were common in both studies, although these two CNAs were observed less often in cases from Finland. However, three prominent CNAs identified in our series [i.e., underrepresentation of 15q11.1–21 (54% of cases), 8p21–p2 (33% of cases), and 3p2 (29% of cases)] were each identified in only 1 of 42 Finnish MMs. In addition, seven cases from our series showed an overrepresentation of 3p12–13, including two cases with an amplification of this region. Gain of 3p12–13 was observed in 2 of 42 MM specimens from Finland (11, 12). The reasons for these discrepancies are unknown but could be attributed to differences in the types of samples examined in the two series; i.e., we focused on cell lines, whereas the Finnish study consisted of tumor specimens. Germaine to this, although underrepresentation of 8p was infrequent in tumor specimens from Finland, small deletions of 8p overlapping band 8p21 were observed in three of six cell lines derived from Finnish tumors (28). Tumor tissues often contain significant amounts of contaminating stromal cells that may mask CNAs. For example, losses of 15q11.1–21 and 3p21 were more apparent in cell line DNA than in DNA from tumor tissue in one of our cases in which an analysis was performed on both sample types (Fig. 1). Discrepancies between the American and Finnish studies could also reflect dissimilarities in the type of asbestos exposure, genetic differences in the study populations, or the possible involvement of a co-factor, e.g., SV40, in the United States that may not be associated with MM in Finland. With regard to the latter, recent studies have demonstrated SV40-like DNA sequences in a significant number of MM specimens from the United States, whereas no such sequences were detected in MM specimens from Finland (16).

In conclusion, the CGH data presented here define a set of genomic imbalances, especially losses of small chromosomal segments, that are a characteristic feature of many MM. The pattern of such losses is consistent with a multistep pathogenetic process involving the loss and/or inactivation of multiple TSGs. Moreover, the delineation of 15q11.1–15 as a new recurrent site of chromosomal loss in MM represents an entry point for the identification of a putative TSG that may contribute to the development or progression of these highly malignant neoplasms.

**REFERENCES**


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